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SEPARATION OF DIASTASE AND PROTEIN FROM WHEAT THROUGH THE ACTION OF SULPHITES

(Third Report)

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It has previously been demonstrated that when wheat meal or wheat flour is stirred vigorously with warm dilute sulphite solutions, a large quantity of "diastase" is set free from the flour, and dissolves in the liquid, where it is active. Furthermore, much of the wheat protein forms a scum containing relatively little starch. When, therefore, the system is allowed to stand after stirring, it settles into three layers: A scum of protein, a middle layer of nearly clear fluid, containing wheat diastase and some other proteins in solution, and a sediment containing starch and the remaining protein.

The diastatic liquid has been used to replace about four-fifths of the malt ordinarily used for conversion of the starch after cooking. It is our understanding that in several alcohol plants where this has been tried the yields of alcohol have remained about the same as with the full amount of malt.

The protein recovered as a scum has been successfully washed with water to remove starch remaining in it. Thereafter it has been denatured by heat and subsequently dried. It has not yet been possible to determine the yield of protein obtained in this way on a large scale. In laboratory experiments the yields have varied from twenty percent of the total protein of the flour up to seventy percent. Yields of fifty percent have been obtained consistently.

In general, wheat flour or wheat meal has been extracted at approximately 40° with 5 times its weight of a sulphite solution varying in concentration from 0.05 to 0.15 percent as sodium sulphite. Vigorous stirring is necessary for the formation of the protein scum, and in the laboratory concurrent aeration has been found advisable. The diastatic extract converts starch to fermentable carbohydrates slowly by itself, but very rapidly in the presence of a small quantity of malt.

The principle of the process appears to be quite sound, but there are numerous factors which affect the yields and qualities of both protein and diastase. While popular interest in this scheme developed very rapidly, we were unfortunately unable for some time to do any laboratory work designed to answer numerous very reasonable queries as to details of the best procedure to be recommended. Such work is now underway again and the following report gives an account of certain experiments done up to this time. The object of these experiments has been first, to study the nature and behavior of the diastatic enzyme extracted from wheat by sulphite and second, to work out conditions tending to a maximum yield of wheat proteins. As a consequence, some useful information about the diastatic enzyme extracted by sulphite solutions is now available, and the yields of protein obtained in laboratory experiments have been greatly improved. Furthermore, wheats previously thought to be totally unfitted for the preparation of protein by this process have been used with good results.

Example of an Experimental Alcohol Mash with Granular Flour

The authors are well aware that the following description does not represent the best way to make these mashes; but it happens to have seemed the best way with the equipment available at the time in the plant in question.

288 bushels of granular wheat flour and one-half bushel of malt were stirred in a cooker with 4500 gallons of water at 100-107° F. containing 40 pounds of anhydrous sodium sulphite and 4 quarts of concentrated sulphuric acid dissolved therein. The pH after the grain had been added was usually about 5.8. Stirring was continued for about 10 minutes after the addition was complete. The mixture was then pumped to an empty fermenter used as a settling tank and a similar batch prepared in the cooker and added to the first. The two batches in the tank were allowed to settle for one or two hours, during which time the protein scum was removed. (Complete protein recovery was not attempted in these mashes.) After settling, about 2000 gallons of nearly clear diastatic liquid was pumped out, and placed in a separate tank where it was cooled to 80° F. The remaining contents of the settling tank were then stirred up, and one half thereof pumped back to the cooker, where it was cooked to 305° F. and cooled to about 155° F. The starch was then converted by the addition of 900 gallons of the diastatic liquor plus 3 bushels of malt. After conversion this mixture was placed in a fermenter and inoculated with the yeast. The second half of the starch suspension in the settling tank was then treated in like manner and after c[illegible] conversion sent to the fermenter to join the first half. The fermenter was then filled to 15,600 gallons with water. The fermentation was started at 70° F., and carried out as usual. The yields by this method have varied from

4.8 proof gallons per bushel of total grain, including that used for the yeast, up to 5.6 proof gallons per bushel when the process got to running smoothly. The average has been slightly over 5.

Evidence for the Proteolytic Mechanism of the Process

The liberation of desmo enzymes by proteolysis, among them desmo amylase from a variety of tissues, was shown by Willstätter. The presence of a powerful but latent proteinase in wheat and also in flour was demonstrated several years ago by this Laboratory. The flour proteinase is activated by the reducing agents applicable to the reduction of any papainase, among them glutathione, cysteine and sulphite. The freeing of amylase from wheat is known to be brought about by these reagents, and also by the proteolytic enzyme, papain. Either proteolysis or reduction of the wheat proteins may thus be indicated. The proof that the mechanism is proteolytic rests on the facts that the liberation of amylase is a time reaction (Fig. 1) and that amylase may also be liberated by trypsin, which is not an enzyme of oxidation or reduction (Table I). The Lintner values of the extracts have here been used as a measure of the enzyme extracted, because, as will appear later, the extracted enzyme is almost entirely of the beta-amylase variety.

The liberation of desmoamylase thus indicates the occurrence of a proteolysis. It is probable that the observed agglomeration of the protein is essentially due to the same cause, but evidence therefor is less clear cut.

Chemical tests detect relatively slight modifications of a protein with only mediocre success. It should be noted that the clotting of a protein is one of the first steps of its hydrolytic breakdown, and that clotted proteins such as coagulated casein show small evidence of proteolysis, if any. Nor is there a good proof that gluten as ordinarily prepared from flour is not already in the clotted stage.

The treatment of flour with sulphite solution results in a greater solubility of nitrogenous material in the solution than would occur in water under similar circumstances. Further, the residual protein in the scum shows signs of being broken down, although to no great extent. Thus, the amino nitrogen is about double that of ordinary gluten but no greater than that of gluten denatured by alkali. Furthermore, the "tyrosine" not precipitable by 0.2 N trichloroacetic acid is slightly but definitely increased when sulphite is added to water suspension of flour. This is a delicate and accepted method of measuring proteolysis. It is unlikely that a profound breakdown of the scum protein occurs, however, for no essential difference in formol titration per unit of protein was found between the original flour and the scum formed by sulphite treatment. Ordinary wheat gluten and the sulphite protein also contained about the same amount of glutamic acid. The data are shown in Table II.

Recovery of Flour Protein in Laboratory Tests

Several factors determine the yield of protein recovered from flour by the sulphite method. Four of these are: Type of stirring, kind of wheat, concentration of sulphite and presence of air. Some deductions as to their several effects on the yield of protein may be made by considering the data presented in Table III. This study is by no means finished.

In the experiments listed in Table III, the flour was extracted (unless otherwise stated) in the proportion of 3500 grams to 18 liters of solution at 42-43° C. The initial pH of the solution after addition of the sulphuric acid was 5.8. The pH of the flour suspension after addition of flour was about 6.1. The stirrer was stopped after the time indicated in the table. The scum was then removed, and puddled (in later experiments sprayed) with cold water to remove admixed starch. Thereafter the protein was heated to 60-65° with a steam jet and filtered out on cheese cloth. The protein on the cloth filter was squeezed to remove loose water, then dried in a current of air at 67-73°, ground in a Wiley Mill and sampled for analysis by Kjeldahl.

The extraction was conducted in a wooden tub of about 8 gallons capacity, equipped with one of two motor driven stirrers, designated in the table as A and B. Stirrer A may be described as producing vigorous but not violent agitation, while in comparison the action of stirrer B was violent. The latter stirrer required a 1/6 horse-power motor equipped with a set of reduction gears to move it at the desired speed through the suspension of flour, water and air. The details of construction will not be discussed because stirring power cannot be described in a way to permit its reproduction in an apparatus of greatly different size.

While on a factory scale agitation alone appears able to incorporate enough air into the mash to cause the scum of protein to rise, this is not the case with the two laboratory stirrers we have used. In these experiments aeration was essential to the recovery of protein, and the amount of air affected the yield. Two rates of air flow were therefore used in different experiments. They are described in Table III as a and b. Rate a was a flow of 1.7-2.5 cc per minute per square centimeter of the surface of the liquid (before the formation of froth). Rate b was 4-5 times as much air for the

first ten minutes of the stirring period, thereafter about the same rate as a. The reduction in aeration was neccssary because otherwise the froth spilled out of the tub.

In considering the data of Table III, the following points appear to be of interest.

It will be noted from runs 3 and 4 that an increase in the temperature up to 50° made very little difference in the protein recovery, but destroyed the amylase in solution. This agrees well with the proteolytic theory of the process.

There appears to be an optimum concentration of sulphite, as shown by runs 13-17. A concentration of 0,05 to 0.10 percent of the extracting medium originally suggested still appears to be the best, but this may well be found eventually to vary with the flour. With no sulphite, no measurable amount of protein was recovered (run 11).

Differences in yield between hard and soft wheats under any one set of conditions are apparent from comparison of runs 1-3 with 5. The yield from soft wheat has always tended to be lower than from hard wheat, but is affected for the better to a greater degree by stirring, air and fineness (runs 5-10).

The question of stirring, air and time of extraction appear to be quite mixed together. More rapid stirring as we did it always incorporated more air. The result of both aerating and stirring as much as possible in our apparatus has led to great improvements in the yield of protein, and shortened the time required to separate it. Thus (runs 3 and 8, also 13 and 18) hard granular flour gave about 5 percent higher yield when treated vigorously. Patent flour gave still greater increases, inasmuch as no protein was recovered (run 20) by the milder treatment (Aa) while a good yield (run 21) was had under condition Bb. Under the latter conditions the highest yields obtained have been with patent flour (runs 9 and 10).

The yields reported here are lower than those of our very first experiments in Washington. There is thus reason to believe that they can be materially bettered after further trials. The handling of soft wheat may, however, present an additional problem that will be considered as soon as time allows.

The protein not recovered as scum was found largely, but not entirely, with the starch fraction. The following experiments (Table IV) indicate that when about 40 percent of the protein was recovered as scum from a medium hard granular flour (Davenport) an equal amount was found in the starch, and the remaining 20 percent was dissolved in the sulphite liquor. The results are based on experiments made as described for Table III; the recoveries of protein have been reported in that table. The results are not exact, since the protein recovered varies from 87 to 96 percent of the total. These inaccuracies are due to the difficulty of sampling the sulphite liquor, which contained suspended matter. This was all centrifuged in the second run, hence the results from it are the more accurate.

Composition of the Crude Protein: The purity of the proteinous materia recovered depends upon the thoroughness with which the starch has been washed out of the scum. The scum from fine flour contains more starch than from granular flour and therefore requires more washing. When this was carefully done in the laboratory a product was obtained in which very little starch remained. Analyses of two such preparations are shown in Table V. The possibility that this material will turn rancid on storage because of the fat content is admitted as presenting a serious problem.

Fate of the Sulphite in the Extraction of Flour

Some of the sulphite disappears when the flour comes in contact with it. This is due to the reduction of oxidized proteins, because it proceeds

with the formation of bodies giving a positive nitroprusside test in the diastatic solution. The reduction is not complete because the test is more intense if the solution is first reduced by alkaline cyanide. Nevertheless, it is clear that the disappearance of sulphite does not necessarily indicate the disappearance of other reducing substances formed by its action.

The disappearance of sulphite in the diastatic liquor appears to be influenced by subsequent aeration, but only to a slight extent. Table VI shows some results on the sulphite remaining in the liquid portion of the system after the addition of flour. The protein recovered as scum in several experiments was likewise tested for sulphite after drying, and was found to be free from sulphite. This again is no indication that the protein is itself not still reduced. Its effect on the properties of bread dough when incorporated therein is good indication that it is reduced. Dr. E. G. Bayfield has reported to us that addition of dried protein to the dough mixture in amounts as small as one percent of dough flour used affects loaf volume and texture adversely.

Observations on the Distribution and Properties of Wheat Amylase

It is known that certainly two, probably three and possibly four or more enzymes may be directly connected with the enzymic hydrolysis of starch to sugars. In order to obtain some useful information about wheat amylase at once, it was decided to restrict the problem by the arbitrary assumption that only two enzymes, so-called alpha- and beta-amylase, are involved. Errors caused by this assumption will resolve themselves into considering as one what in reality may be a mixture of two or more catalysts. For the purposes of the present work, this is not fatal; but we will make an attempt later to clear up some of the uncertainty that now surrounds the whole amylolytic system.

Estimation of Alpha- and Beta-Amylase

On the assumption that "diastase" consists of only 2 enzymes, namely alpha- and beta-amylase, the following procedure was adopted for estimating the quantity of each. The methods are modifications by Dr. S. Schwimmer of methods already described in the literature. They are based on the fact that beta-amylase digests about 64 percent of (potato) starch to an "amylodextrin" which is resistent to further action by beta-enzyme, but is acted upon by alpha. The amylodextrin gives a purple-red color with iodine, which disappears during the action of alpha-enzyme upon it. By measuring the rate of disappearance of the purple color in a sample of starch already acted upon by an excess of beta-amylase, and comparing this rate to that observed with saliva, which is nearly pure alpha-amylase, an estimation of alpha-amylolytic activity can be had.1/

By measuring the amount of sugar formed from starch solution by a mixture of the enzymes in a given time and correcting this amount to allow for the sugar forming power of the (already determined) alpha-amylase present in the mixture, the sugar formed by the beta-enzyme alone may be found, and used as a measure of that enzyme.

Reagents used:

1. Stock Iodine Solution - 11 g. iodine + 22 g. KI to 500 cc. with water.

2. Dilute Iodine Solution - 20 g. KI + 2 cc. Stock Iodine to 500 cc. with water.

3. Two percent boiled Lintner starch containing 0.02 M acetate pH 4.8.

1/ The plant alpha-amylase is thus measured by comparison with an animal amylase, a process that is not without possibility of error, though it has given very consistent results for our purposes.

4. Beta-amylase solution, prepared by stirring 40 g. of whole hard wheat meal with 100 cc. of water at room temperature, then centrifuging.

5. Reagents for maltose determination, as directed by Cereal Laboratory Methods, A.A.C.C., 4th Ed. 1941, page 95. These are : Alkaline ferricyanide solution, sodium thiosulphate, acetic acid-salt solution, potassium iodide solution. (The NaOH solution, intended to stop the enzyme action, was not used by us.)

Estimation of Alpha-Amylase:

A series of test tubes was prepared, each tube containing 5 cc. of the dilute iodine solution (reagent 2).

20 cc. of starch solution was warmed to 30° C. and 2 cc. of beta-amylase solution (reagent 4) was added thereto. After exactly 10 minutes incubation, the alpha-amylase sample, diluted to 8 cc., was added to the partly digested starch. Thereafter, at appropriate time intervals (of perhaps 1 or 2 minutes) 0.5 cc. portions of the mixture were removed and mixed with the iodine solution in one of the test tubes already prepared. The color of the iodine solution placed in a quarter inch photometer cell was read immediately in a neutral wedge (visual) colorimeter using a filter for 540 mμ.

The results plotted against time of digestion were used to determine by interpolation the time required to produce a color that gave a colorimeter reading of 40.[2/] This time was then referred to a curve (Fig. 1), showing

2/ To establish the corresponding reading in another type of colorimeter, the following aqueous mixture, which keeps for a couple of hours at room temperature may be used to reproduce the color: Dextrin (Eastman, bacteriological, 0.066 percent, Potassium iodide, 3.1 percent, Iodine (added last), 0.118 percent.

If a more dilute colored solution is required, it may be obtained as follows: Dextrin (Eastman, bacteriological), 0.086 percent, Potassium iodide, 3.1 percent, Iodine, 0.00176 percent.

The reading of the weaker solution in our instrument corresponds to that of the stronger solution when the volume of the latter prepared in the test (i.e. 5.5 cc.) is diluted to 25 cc. with water and then read.

time vs. arbitrary units of alpha-amylase, prepared by this procedure with dilute saliva as a source of alpha-enzyme.

One unit of alpha-amylase was taken as that amount of enzyme (as saliva) that caused the starch solution to produce the standard color with iodine (scale reading of 40) in 10 minutes under the condition just described.

This scale reading has been selected because the change in color per minute in that range is roughly a maximum. Fig. 2 shows examples of the change in colorimetric readings with time at different concentration of alpha-amylase.

When the quantity of alpha-amylase is large it may be calculated, as shown by Fig. 3, by the linear relationship between alpha-amylase units and the reciprocal of the time required to reach the standard color. Thus

$$\text{Units} = 0.32 + \frac{6.8}{t\ (\text{min.})}$$

When, however, the quantity of alpha-amylase is less than one unit in the total digest, it is necessary to use the curve connecting units with reciprocal times (Fig. 3).

The units have been uniformly expressed as the total in the digestion system, here 30 cc., consisting of 20 cc. of starch solution, 2 cc. of beta-amylase preparation and 8 cc. of the alpha-amylase preparation under test.

Determination of Beta-Amylase: 20 cc. of starch solution were mixed with 10 cc. of enzyme solution in water at 30° C. and incubated for 10 minutes. Thereupon a 3 cc. sample of the mixture was removed and added to 10 cc. of 0.05 N potassium ferricyanide solution. The latter mixture was placed in a boiling water bath for 20 minutes, cooled, 25 cc. of acid-salt mixture (as in reagent 5) and 1 cc of 50 percent KI solution were added thereto, and the final mixture titrated as usual with 0.05 N thiosulphate solution.

One unit of beta-amylase was taken as the quantity of enzyme in the original 30 cc. total system (i.e. the quantity taken as a sample at the start) that would have reduced 100 m.eq. of ferricyanide in 10 minutes. What is measured is one tenth of this amount.

The results were calculated as follows:

(cc. thiosulphate used in blank titration - cc. in test titration) x Normality of thiosulphate = m.eq. (of ferricyanide reduced) corresponding to the sugar in the aliquot taken.

M.eq. corresponding to sugar found - M.eq. due to alpha-amylase (Fig. 4) = M.eq. in aliquot due to beta-amylase.

M.eq. in aliquot due to beta-amylase x 10 = m.eq. in enzyme sample used.

The relation between m.eq. of ferricyanide and units of beta-amylase is shown in Fig. 5.

This procedure is quite accurate, because the sugar formed by the alpha-enzyme is small in comparison to that formed by the beta. Furthermore under our conditions, the sugar produced by the beta-enzyme is independent of the sugar produced by the alpha-enzyme present along with it, as shown in Table VII.3/

3/ The methods just described may encourage the suspicion that the alpha-amylase of a wheat extract will be found to be low because it is measured by comparison with a similar wheat extract assumed to contain no alpha-amylase. This, however, is not the case, because the addition of known and relatively large quantities of alpha-amylase have been found in independent determinations to produce but little sugar, and thus proving that the assumption is justified. As far as our sulphite extracts are concerned, this should have been sufficient evidence. The saccharifying power, as shown by the Lintner value, could be used as a measure of total enzyme, as has been done in other experiments. The advantage of an estimation of alpha-amylase is only apparent when the question arises of mixtures of wheat amylase with preparations high in alpha-amylase.

Nature of Amylase in Sulphite Extract

By the aid of the methods just outlined, it was found that the amylase extracted by sulphite contains very little alpha-amylase. This is apparent because of the behavior of the sulphite extract after the addition of small extra quantities of alpha-amylase. Thus, it may be seen from Table VIII that barley malt contained roughly 200 times as much alpha-amylase as did wheat flour. A preparation of mold bran contained three times as much alpha-amylase as barley malt, and an old sample of pancreatin contained 16 times as much.

An amount of malt equivalent to 2 percent of the wheat flour has been found in practice to accelerate the conversion of starch by wheat diastase markedly and sufficiently. The relative proportions of alpha- and beta-amylase in such a mixture have therefore been found; also the amount of pancreatin required to give the same mixture of these enzymes.

Amylase Content of Sulphite Extracts

The sugar forming enzyme, as measured by the Lintner value of the extract, was found to vary greatly with the kind of grain and also with the variety of wheat. Table IX shows the Lintner values obtained when an extract was made from 100 g. of material in 400 cc. of 0.05 percent Na_2SO_3 at 45° C. for 1 hour.

Effect of the Concentration of Flour on Extraction of Amylase

The following experiment on Hampstead (1942) granular flour indicates that the proportion of flour to extractant may vary within wide limits without appreciable loss of diastase in the extract (Table X). It should be remembered however, that less extract can be conveniently removed from the sediment as the proportion of flour to extractant is increased. On the other hand, because of the greater protein content of the more concentrated extracts, the enzyme therein is more stable, as might of course be expected.

Stability of Diastase Extracted by Sulphite

Because of the small amount of alpha-amylase present, the sugar-forming property of the solution has been taken as a criterion of active enzyme present, and has been measured by the simple Lintner-value technique. The following observations (Table XI) show the effect of temperature and pH on the enzyme remaining active after three hours. Storage of sulphite liquid for 2 days at 23° C. also produced a decided loss, namely from 82 to 41 Lintner units. This extract was made with 0.05 percent sodium sulphite, and the probability that infection was the cause of this loss is good.

The effect of formaldehyde, proposed by Mr. P. A. Singer, as an agent to reduce infection may therefore be important. The enzyme extracted by

sulphite solutions is sensitive to large but not to small doses of formaldehyde, as appears from Table XII. This was shown by short exposure of the enzyme to the reagent. The effect on infection, as evidenced by long exposure was not studied.

The Condition of Beta-Amylase in Flour

Evidence has been presented to show that the extraction of amylase from flour by sulphite solution is due to a proteolysis. The enzyme protein must thereby be changed in one or both of two ways: Transformed from an insoluble to a soluble protein, or from an inactive to an active one. The following experiments indicate that the enzyme exists in flour in both an active and an inactive state. The inactive form is soluble in neither water nor NaCl solution to any marked extent.

The experiments consisted in a comparison of the amylolytic power extracted successively from flour by water, NaCl solution and papain. The total beta-amylase present in the flour was estimated by measuring the amylase after treatment with active papain, where the quantity of papain was sufficient to dissolve practically all of the flour protein. This total, as shown in Table XII, is in good agreement with the sum of the various fractions measured separately. After papain digestion all the amylase must be considered as in solution and also active. The amylase obtained with sulphite is usually 75-85 percent of the total.

The activation of the inactive amylase is also a proteolysis, for it ca. be produced by trypsin as well as by papain. This observation points to the inactive enzyme as being a true proenzyme protein.

125 g. of unbleached granular flour (Davenport, 1943) was extracted for 30 minutes with 500 cc. water at 40°. The suspension was centrifuged slowly

and the residue washed at once with water in about the same proportion as before, and centrifuged out again. After the water extracts were combined, the content of beta-amylase therein was determined by the method here described, and also determined after the addition of a large dose of naturally active papain thereto.

A second series of two extractions was then made with 5 percent NaCl, in the same manner as the first series with water.

The residue remaining after washing with salt solution was then suspended in a known volume of water, and aliquots of this suspension were then used for the determination of amylase before and after digestion with papain.

An exactly similar series of experiments was then run on the same flour after after it had been ground for a week in a ball mill. The resulting powder was extremely fine.

It appears from Table XIII that most of the amylase not soluble in NaCl solution was also inactive until treated with papain. In spite of the general experience that enzymes are active while insoluble, and because of the large particles in granular flour, it is not safe to assume that the inactive amylase in this case was not simply buried inside the particles, and liberated when they were dissolved by the proteinase. Such, however, is not the case; for if it were, the proportion of inactive enzyme in the impalpably fine ball-milled powder would be very small indeed. While the grinding destroyed nearly two-thirds of the total amylase of the flour, it is evident from Table XIV that it did not greatly disturb the proportion of inactive to active enzyme remaining. A small difference in the stability of the enzyme and proenzyme proteins toward grinding may be reasonably expected.

It is therefore evident that the beta-amylase of wheat exists in the natural state in two forms, an active and an inactive form. The active form is partly soluble in water and almost entirely dissolved by dilute salt

solution. The inactive form is not readily soluble in water or salt solution, and is changed to a soluble active enzyme by treatment with papain, sulphite or trypsin.

Table I

Extraction of Amylase by Several Reagents

(Granular flour (Davenport, 1943) was stirred with 5 times its weight of solution for 60 minutes at 40-45° C.)

Extractant	Lintner Value[1] on Clear Extract
0.05 percent Na_2SO_3	140
Commercial trypsin 500 mg/l.[2]	114
Crystalline trypsin 150 mg/l.[3]	87
Crystalline trypsin 1500 mg/l.	80
Crude papain (10.0 g/l.)[4]	160
5.0 percent NaCl	77
Water	40

1/ Determined as directed by Cereal Laboratory Methods, A.A.C.C. 4th Ed. 1941, p. 94, except for method of extraction, which was as stated in each case. The Lintner value is a satisfactory measure of amylase because of the low content of alpha-amylase present in these extracts.

2/ Trypsin had no amylolytic activity.

3/ About 50 percent active protein.

4/ About 30 percent active protein; had no amylolytic activity.

Table II

Comparison of Protein in Flour with Sulphite Extracted Protein and with Ordinary Wheat Gluten

	Amino N[1] %	Glutamic Acid[1] %	Tyrosine Color Value on Acid Filtrate[2]
Sulphite protein (hard wheat flour)[1]	0.23	38-40	
Wheat gluten	0.14	38-40	
Patent flour			
a. suspended in H_2O			14.8
b. suspended in 0.075 Na_2SO_3			15.2
Patent flour first washed with water, then:			
a. suspended in H_2O (30'; 42° C.)			6.55
b. suspended in 0.075% Na_2SO_3 (30'; 42° C.)			8.38

1/ Courtesy of Western Regional Laboratory.

2/ M.eq. x 10^4 of tyrosine per 5 cc. trichloroacetic acid filtrate, representing 0.039 g. of flour protein.

Table III

Yields of Wheat Protein

Run #	Kind of Flour	Protein in Flour: N x 5.7 %	Conc. of Na_2SO_3 %	Lintner Value of Extract	Time of Extraction min.	Crude Protein Recovered g.	Yield of Pure Protein	Stirrer	Air	Notes
1	Sperry, 1943, newly ground, hard, gran. lot 1	14.4	0.05	93	145	835	48.3	A	a	1
2	Ditto	"	"	103	90	640	39.4	A	a	1
3	Ditto	"	"	89	90	732	41.1	A	a	1
4	Ditto	"	"	57	90	660	36.2	A	a	1-2
5	Sperry, 1943, soft, gran., lot 2	9.35	0.075	52	60	132	28.6	A	a	
6	Ditto	"	0.10	-	45	209	39.1	B	b	
7	Ditto	"	0.075	-	60	180	43.3	B	a	
8	Sperry, 1943, hard, gran., lot 3	12.05	0.10	-	30	259	46.4	B	b	
9	" " " , pat., lot 4	13.16	"	-	30	335	58.4	B	b	
10	" " " " , lot 6, bleached	13.43	"	-	30	321	55.6	B	b	
11	" " " " , lot 3	12.05	none	-	45	trace	-	B	b	
12	" " " " , lot 6, bleached	-	0.075	-	45	none	0	A	a	
13	Davenport, 1943, gran.	11.8	0.075	88	60	245	37.8	A	a	

Run #	Kind of Flour	Protein in Flour N x 5.7	Conc. of Na_2SO_3	Lintner Value of Extract	Time of Extraction min.	Crude Protein Recovered	Yield of Pure Protein	Stirrer	Air	Notes
		%	%			g.	%			
14	Davenport, 1943, gran.	11.8	0.150	-	60	80	14.9	A	a	5
15	Ditto	"	"	-	60	193	32.9	A	a	
16	Ditto	"	0.225	-	60	132	24.1	A	a	6
17	Ditto	"	0.050	-	60	162	32.1	A	a	
18	Ditto	"	0.10	-	50	249	43.0	B	a	5
19	Ditto	"	0.075	116	45	194	33.3	A	a	4
20	Kansas, 1942, hard, pat.	12.35	0.10	-	45	trace	0	A	a	
21	Ditto	"	"	-	45	351	55.5	B	b	
22	Whole wheat meal, north, hard	15.5	0.075	34	60	169	21.2	A	a	3
23	Ditto	16.1	0.10	-	40	217	25.5	B	b	
24	Kansas, 1942, hard, pat.	12.35	"	-	45	357	56.2	B	b	
25	Ditto	"	"	-	30	336	54.0	B	b	

1. 5 Kg. flour in 18.9 l. of solution.
2. Temperature of extraction 50° C.
3. Protein washed first in cold water, then heated as described.
4. Lintner value not on this run, but on a similar extract.
5. No air for first ten minutes of stirring.
6. Sample washed and dried in 2 portions.

Table IV

Nitrogen Balance Experiments

(3500 g. of flour, 18 liters of sodium sulphite solution, 0.075% at pH 5.6; temp. 41-3°; time 45 min.; wash water about 4 l.)

Flour	Protein in flour %	Percent of the Protein found in				Sum of percentages recovered	Notes
		Scum	Sulphite	Wash water	Starch		
Sperry (lot 2)	9.35	28.6	38.2	3.2	17.4	87	(1)
Davenport	11.8	37.8	13.1	3.1	42.2	96	(2)

(1). Suspension settled very slowly, and protein ordinarily found in sediment was measured as though in solution.

(2). Sediment and liquid fractions recovered by centrifuging the entire batch.

Table V

Composition of Crude Protein

	(See Table III) Run # 9 %	Run # 10 %
Yield as pure protein	58.4	55.6
Crude protein contained:		
protein	80.3	81.3
fat	0.6	0.8
moisture	6.2	8.3
starch (by diff.)	12.9	9.6
Total	100.0	100.0

Table VI

Sulphite in Diastatic Liquid and in Protein

(Immediately after mixing flour and solution as usual and stirring for 45' at 40-43°)

Conditions	SO_2 found[1]	
	Mg. per cc.	Fraction of SO_2 added
		%
Original Na_2SO_3 Solution before adding flour	0.45	100
Diastatic liquid; extract with 0.1% Na_2SO_3 3 min. after mixing	0.31	68.0
Diastatic liquid; heavy aeration for 45' in presence of protein scum	0.29	64.0
Diastatic liquid; heavy aeration for an additional 60' after removal of protein scum	0.23	51.0
Dried protein; heavy aeration, from gran. flour	-	0
Dried protein; heavy aeration, from unbleached patent flour	-	0
Dried protein; heavy aeration, from bleached patent flour	-	0

1/ Method of A.O.A.C., 5th Ed. (1940) pg. 463; but sulphate weighed as $BaSO_4$.

Table VII

Showing Independent Action of Alpha- and Beta-Amylases

(Procedure as outlined under Methods)

cc of beta solution (wheat extract)	M.eq. x 10^2 of ferricyanide reduced in the total digestion mixture: without added alpha	in presence of 1 unit of alpha	excess due to alpha enzyme	corrected for sugar formed by 1 unit of alpha
0	-	6	6.0	-
0.05	73.5	80	6.5	74
0.06	80.5			80.5
0.15	193	198	5.0	192
0.30	315	323	8.0	317

Table VIII

Proportions of Alpha- and Beta-Amylases in

Sulphite Extract, etc.

Extracted by	Enzyme Units per Mg. of Flour Extracted	
	Alpha	Beta
Water	.00010	.025
.05% Na_2SO_3	.00013	.10
.05% Na_2SO_3 + 2% malt[1]	.00065	.10
.05% Na_2SO_3 + 0.13% commercial pancreatin[1]	.00065	.11
Barley malt (water extract) (a)	.0264	--
" " " " (b)	.0158	.04
Mold bran (oryzae)[2] (water extract)	.060	.01
Pancreatin (water extract)	.40[3]	.07
Saliva	.86[3]	--

1/ The malt and pancreatin given in percent of flour used in the extraction.

2/ Courtesy of Dr. E. I. Fulmer, Iowa State Experiment Station.

3/ Units per mg. of total solids.

Table IX

Diastatic Activity of Sulphite Extracts of Various Cereals

(0.05% Na_2SO_3; 45°; 1 hr.)

Material	Lintner Value[1]
Granular flour (Hempstead, 1942)	140
" " (local, 1943)	90
" " (Davenport, 1943)[2]	140
Rye - whole meal (probably local, 1943)[2]	75
Wheat bran, coarse	85
" " , fine	90
Wheat chaff	trace
Kaffir corn, whole meal (local, 1943)	trace
Whole wheat meal (local, 1943)	70
Patent flour (Kansas hard wheat, 1943, bleached)	73

1/ The proportion of grain to extract is that given here, not that used in the Lintner determination. Results calculated to a per-gram basis, as in all cases.

2/ Without sulphite, 40.

Table X

Variation in Flour Concentration

(Extraction with 0.10% Na_2SO_3)

	Linter value of extract (expressed per gram of flour used.)	
	Extract of 100 g. flour with 400 cc. solution	100 g. flour with 200 cc. solution
Fresh extract	140	130
After 3 hrs. at 50°	75	130
Fresh extract + NaClO (0.008%)		83
After 3 hrs. at 50° with added NaClO		82

Table XI

Effect of pH and Temperature on Lintner Value of Sulphite Extract

(Made with neutralized 0.05% Na_2SO_3 solution)

pH	Lintner value after 3 hours at:						Control (no storage time) (pH 6.5)
	25°	40°	45°	50°	56°	65°	
5.0	133	95		53			133
5.5	153	144		71			133
6.0	136		131	67	41	7	133
6.5	144	141		68			133

Table XII

Effect of Formaldehyde on Lintner Value of Diastase

Extracted by 0.05% Na_2SO_3

(Flour digested with sulphite 30 min. at 35°; formaldehyde added; test made after 10 minutes longer at 35°)

	Formaldehyde % at final vol.	Lintner
Whole flour suspension	0	107
	0.01	103
	0.02	100
	0.04	99
Sulphite solution extract	0	107
	0.01	105
	0.03	106
Formaldehyde added to sulphite solution before flour	0	107
	0.01	98
	0.20	19

Table XIII

Extraction of Beta-Amylase from Flour

(Units refer to the enzyme in the total sample, 12.5 g.)

Fraction	Granular Flour			Ball Milled Flour		
	Beta-Amylase Units		% Activity	Beta-Amylase Units		% Activity
	Active	Total		Active	Total	
Extracted by water	280	337	83	135	167	81
Extracted thereafter by 5% NaCl	282	306	92	78	98	100
Residual enzyme	173	918	19	144	339	43
Sum	735	1561	47	357	584	61
Total enzyme: Measured on suspension after direct digestion of flour with papain (no previous extractions)		1648			583	
Enzyme unaccounted for		+87			-1	

Table XIV

Properties of Beta-Amylase in Flour

Condition of Enzyme	Percent of Total Enzyme	
	Granular Flour	Ball Milled Flour
Soluble in water		
a. active	17	23
b. inactive	4	5
Not soluble in water, but soluble in 5% NaCl		
a. active	17	13
b. inactive	1	0
Not soluble in 5% NaCl		
a. active	11	25
b. inactive	45	34
Unaccounted for	5	0
Total	100	100

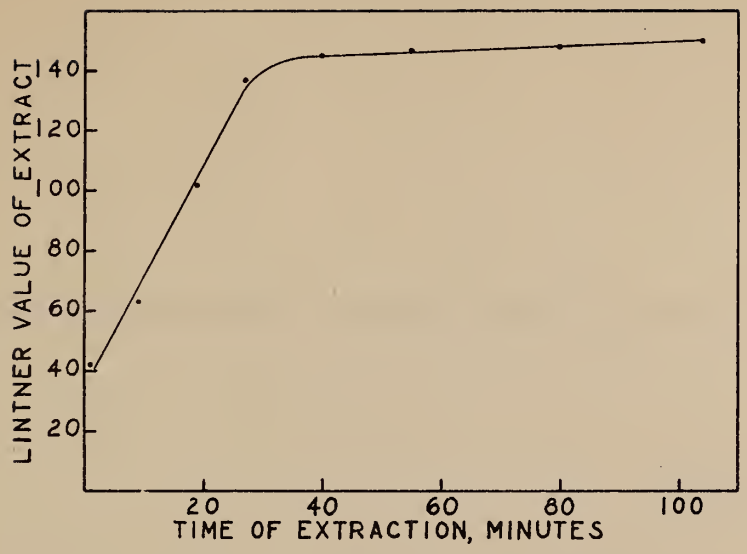

Figure 1. Showing the extraction of amylase by sulphite to be a time reaction. (100 g. unbleached granular flour in 500 cc. of 0.05 % sodium sulphite at 40° C.)

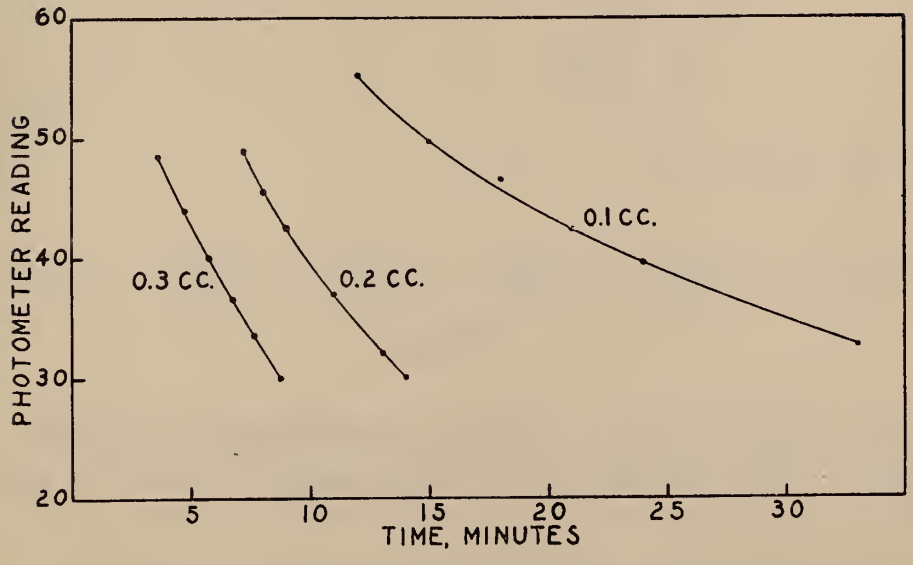

Figure 2. Showing the color change observed with 3 different amounts of salivary amylase.

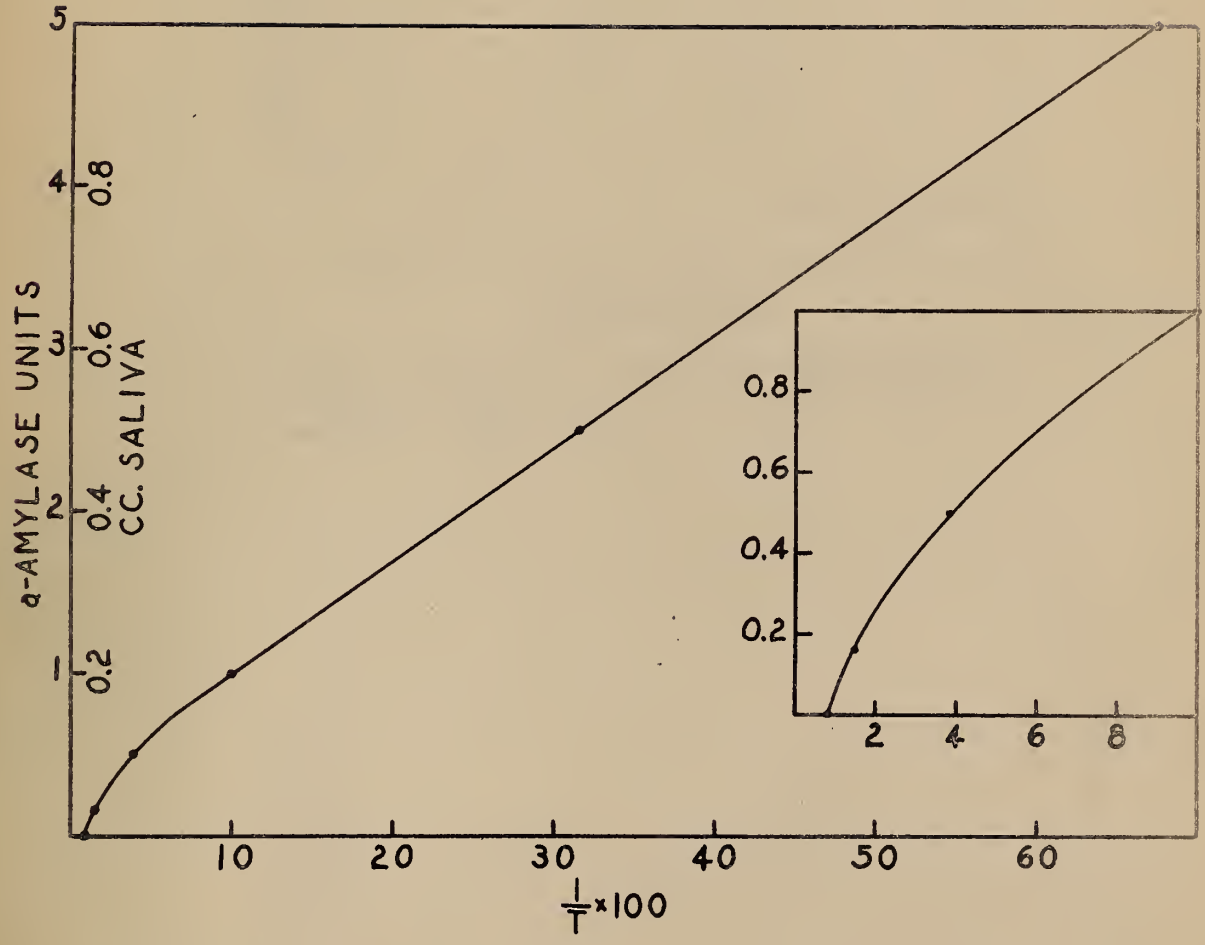

Figure 3. Showing the relationship between alpha-amylase units and time required to reach standard color. The inset is on a larger scale.

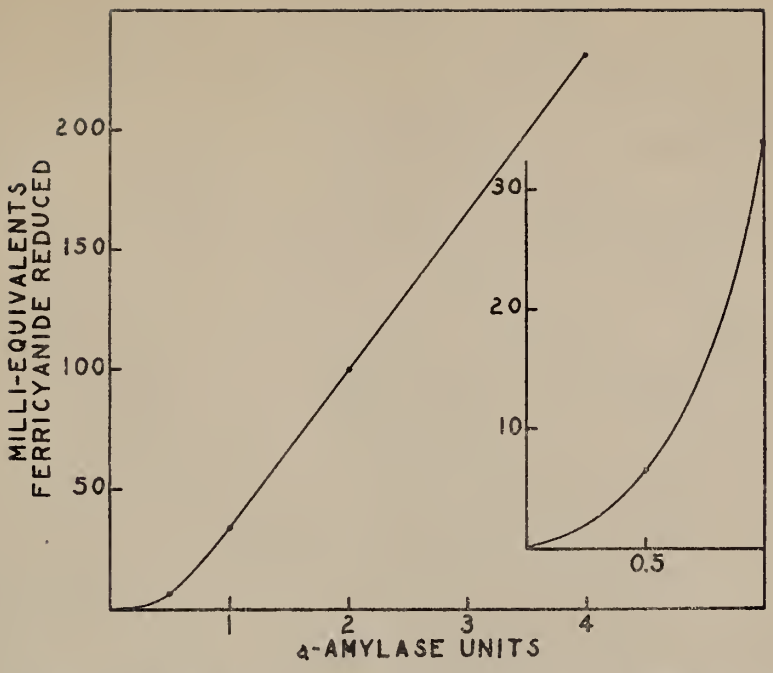

Figure 4. Showing the correction for alpha-amylase expressed as milliequivalents of ferricyanide reduced. The inset is on a larger scale.

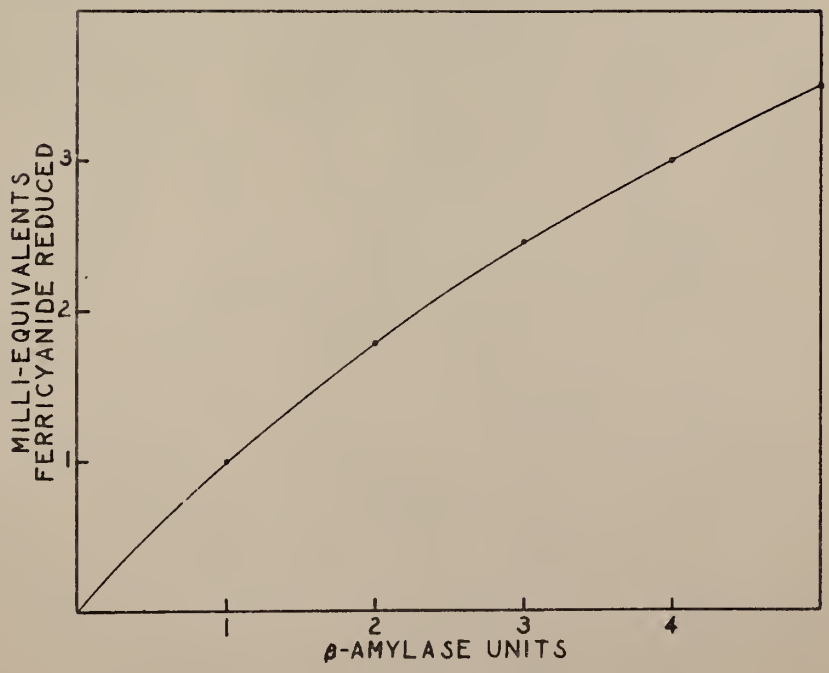

Figure 5. Showing relation of beta-amylase units to milliequivalents of ferricyanide reduced.